# THE GUM AND BYPRODUCTS OF *BACTERIUM SACCHARI.*

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In May of last year I read a paper before the Society upon "An Ascobacterium from the Sugar-cane, with Notes upon the Nature of the Slime."* The chemical notes regarding the slime were of a preliminary nature, and showed that the slime yielded a carbohydrate containing some nitrogenous impurity. Under certain conditions of preparation, the carbohydrate, which may be called a gum, was soluble in water and was readily converted into an insoluble modification by treatment with alcohol. The gum yielded furfural on treatment with hydrochloric acid, and gave a reducing sugar upon hydrolysis with dilute sulphuric acid. The osazone with the melting point of 153° which was obtained was, in view of my later researches, probably contaminated with a substance that reduced the melting point. At that time methods for the purification and separation of mixed osazones had not been described, and the difficulty of obtaining the slime in quantity had militated against my devising a method for the purification. Since then, however, I have so improved not only certain media for growing gum-producing bacteria, but also the methods for purifying and separating the osazones of arabinose, galactose and glucose. A small quantity of carbohydrate is now sufficient to enable a determination of the products of the hydrolysis to be made with a considerable degree of

* These Proceedings, 1903, 137 *et seq.*

precision. To complete my work upon *Bact. sacchari*, I determined to reinvestigate the slime.

Experiments with other slimes had led to the preparation of a medium containing potato juice 100 c.c., glycerine 50 grm., tannin 3 grm., agar 20 grm., and tap-water to make a litre. A preliminary sowing of *Bact. sacchari* upon a plate of this medium showed that it produced a luxuriant slime which did not adhere to the medium. In view of this favourable result, large plates of the medium were sown with the bacteria. The most convenient size of Petri dish measures $15 \times 2$ cm., and easily holds 100 c.c of agar medium. When larger dishes are used there is always too much condensation of moisture upon the cover. The drops of water that gather fall into the solidifying medium which is softened locally and the soft agar is removed with the slime. The infected plates were maintained at the laboratory temperature (18°-20°). Upon the fifth day 135 c.c. of a thick slime were removed, two days afterwards another 58 c.c. were gathered, and on the tenth day another 20 c.c., making a total of 213 c.c. of slime which had been obtained from a litre of medium.

The slime was freed from glycerine and other matters by precipitation with alcohol, resuspension in water followed by a second treatment with alcohol. As the slime was acid and coagulation with the alcohol was not complete, it was nearly neutralised with potassium hydrate. Neutralisation to phenolphthalein or to litmus caused a darkening of the slime from the tannin contained in it, so care was taken to maintain the slime just sufficiently acid to prevent any prominent change of colour. The slime was rather deficient in saline matter, as was evidenced by the alcohol producing a "milk," but the addition of potassium chloride and the warming of the alcoholic fluid induced a complete coagulation.

After the second coagulation, the slime was tested for reducing sugars, and as none were found the coagulated slime was treated with water until a homogeneous emulsion was obtained. This was heated on the water-bath to expel the bulk of the small quantity of alcohol that had adhered to the coagulum. The

emulsion was then heated in the autoclave at a pressure of three atmospheres for fifteen minutes. This treatment produced a separation of the slime into a comparatively clear supernatant liquid and a sediment. The sediment was treated with water and again heated in the autoclave. The second heating had apparently brought all the remainder of the gum into solution, for the insoluble matter was not at all slimy. The gum solutions were clarified with a little aluminium hydrate and, after filtration, concentrated by evaporation. About 100 c.c. of a thick, viscous, transparent gum mucilage were obtained. This was adhesive, and firmly fastened paper to glass.

Upon testing drops of the thick gum mucilage with drops of reagents upon a glass plate as recommended by Maben,* basic lead acetate and ammoniacal lead acetate gave white curdy masses, ferric chloride gave a translucent brownish clot, barium hydrate thickened the mucilage, Schweitzer's reagent produced a gelatinous slime, dilute iodine gave a reddish tinge; no reactions were obtained with borax paste, copper sulphate, neutral lead acetate, milk of lime, aluminium hydrate, potassium hydrate, or sodium silicate. The precipitation with lime water was not confirmed. Copper sulphate followed by potassium hydrate gave a gelatinous blue precipitate which contracted to a curdy mass upon boiling. Fehling's solution under similar conditions gave no coagulation—a point wherein the gum differs from many others, *e.g.*, yeast gums.†

A portion of the gum was boiled with 5 % sulphuric acid for five hours, when portions showed, upon being tested, the absence of gum and the presence of reducing sugars. After removal of the sulphuric acid by barium hydrate, the osazones of the sugars were prepared. They were obtained fractionally by the repeated addition of phenylhydrazine acetate solution followed by heating on the water-bath. Three fractions were obtained. These were,

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* Journ. Pharm. xx., 719.

† Lafar, Technical Mycology, ii., 1, 178.

in great part, freed from tarry bodies by moistening with alcohol and treatment with ether.

The three fractions were separated into a number of portions by means of (1) warm water, (2) solution in hot alcohol and cooling of the solution, and (3) evaporation of the alcohol. All the fractions contained galactosazone and a small quantity of a vitreous yellow impurity which melted at 150°. The latter had undoubtedly been present in the osazone of my earlier research. No osazone other than galactosazone was obtained.

Thus the carbohydrate of *Bact. saccharı* is a galactan. It had been shown to give the furfural reaction, and in confirmation of its nature it was found to yield mucic acid upon oxidation with dilute nitric acid.

Galactan was also produced by the bacterium in fluid saccharose cultures, and especially was the presence of the slime shown when the medium contained chalk. In these solutions there was no production of reducing sugars, so that the organism did not secrete invertase. Acids were produced from saccharose, and the identification of these was necessary to complete the investigation. The medium in which they were formed consisted of saccharose 50 grm., peptone 5 grm., potassium phosphate 1 grm., potassium chloride 5 grm , chalk 10 grm., and water 1000 c.c. The method of separating the acids was essentially that described on pp. 118-120 of these Proceedings.

The ratio of the volatile to the non-volatile acids was as 8·1 : 34·7, or roughly as 1 : 4. The former consisted of acetic and formic acids. Acetic acid was identified by the silver salt and the odour. Formic acid was proved by the blackening of the filtrate from the silver acetate and the formation of calomel upon boiling the solution of the acids with mercuric chloride. The non-volatile acids consisted of succinic with small quantities of lauric and palmitic. The first was identified by its melting point, capability of being sublimed, and by the formation of the ferric salt. The separation of the lauric and palmitic acids, which separated as a fat after removal of the ether, was effected by warming the mixture upon porous porcelain at 45° for some

hours. The residue on the porcelain melted at 61° (palmitic acid m.p. 62°) and that absorbed by the porcelain and recovered from it melted at 44° (lauric acid m.p. 43°).

During the fermentation of saccharose, carbon dioxide was evolved. This was shown by drawing the air in small culture flasks through baryta water. The method of procedure has been described on page 548 of these Proceedings. Ethyl alcohol was also produced. This was separated from the culture media in the manner described on page 344.

*Summary.*—In media containing saccharose, *Bact. sacchari* produces a galactan gum, carbon dioxide, ethyl alcohol, lauric, palmitic, succinic, acetic and formic acids.